The Analysis of Polar Pesticides by Ion-Exchange Chromatography Tandem Mass Spectrometry; A Tale of Two (and many more) Molecules

Dr Stuart Adams

Presentation overview

- Quick introduction to Fera Science Ltd
- Polar Pesticides and Quick Universal Polar Pesticide Extraction (QuPPe) method
- Ion chromatography mass spectrometry instrumentation
- Results of current methods and validations
- Summary

Fera Science Ltd (www.fera.co.uk)

- A leading supplier of scientific solutions, evidence and advice across the Agri-Food supply chain in Europe – “from farm to fork”

100 years of science

600 R&D projects

100,000 samples a year

1000 active collaboration partners

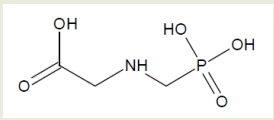
National Reference Laboratory

GLP / ISO Compliant

Emergency contingency capability

Polar Pesticides: old news?

- Glyphosate – approval in the EU currently in question
  - In 2015 World Health Organisation moved to classify glyphosate as a probable human carcinogen



- Ethephon – approved but frequent MRL violations
  - Current 2016 figures from RASFF, 4 alerts and 3 information for attention notifications covering, grapes, tomatoes, peppers and figs

- Chlorate – banned by EU in 2010 because of health risks
  - EFSA called for more data on the presence of chlorate in foods

- Perchlorate – EU established MRLS of 10 µg/kg for most foods in 2015
  - Dietary exposure study done by U.S. FDA – milk contributes to about 47% of total exposure (FDA website, www.fda.gov)

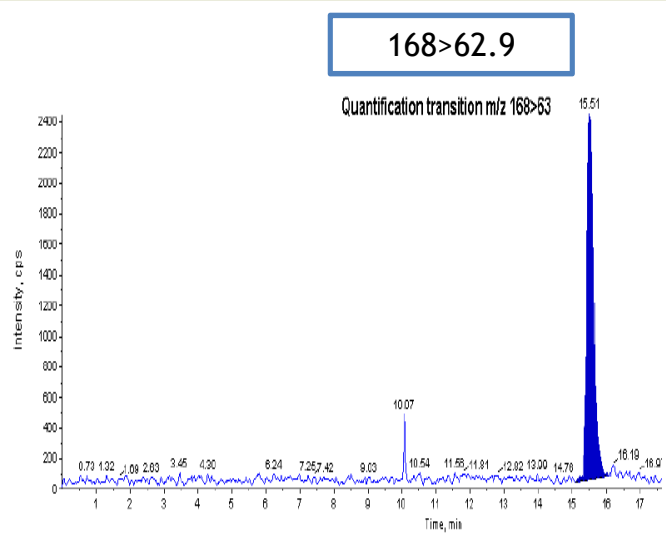
QuPPe – The Perfect Extraction Method?

- Generic extraction using acidified methanol – no partition, no clean-up
- The QuPPe method developed by EURL-SRM is not perfect
  http://www.eurl-pesticides.eu/docs/public/tmplt_article.asp?LabID=200&CntID=1005&Theme_ID=1&Pdf=False&Lang=EN
- The Cons:
  - Extracts contain high amounts of co-extractives
  - Variation in retention time
  - Internal standards required
  - Several different column chemistries required

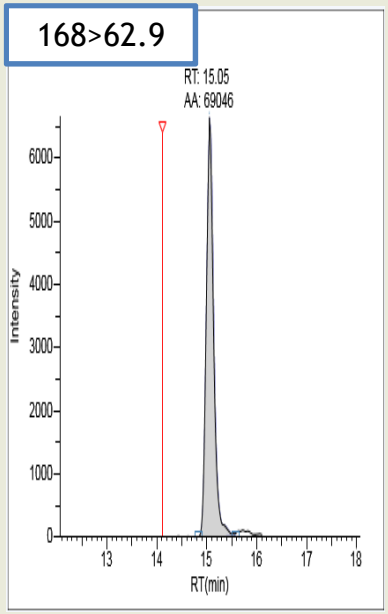
- The Pros:
  - Continually evolving (current version (9) released in March 2016)
  - Cost effective compared to previous approaches
  - QuPPe has enabled analysis of pesticides monitored infrequently in the past
  - Labelled internal standards have become commercially available

IC-MS/MS then and now - glyphosate

- 2007
  Glyphosate @ 100 µg/kg in cereals with 2.5 mL injection online concentration



- 2016
  Glyphosate @ 100 µg/kg in cereals with 1/10 extraction dilution of QuPPE extracts, 100 µL loop injection

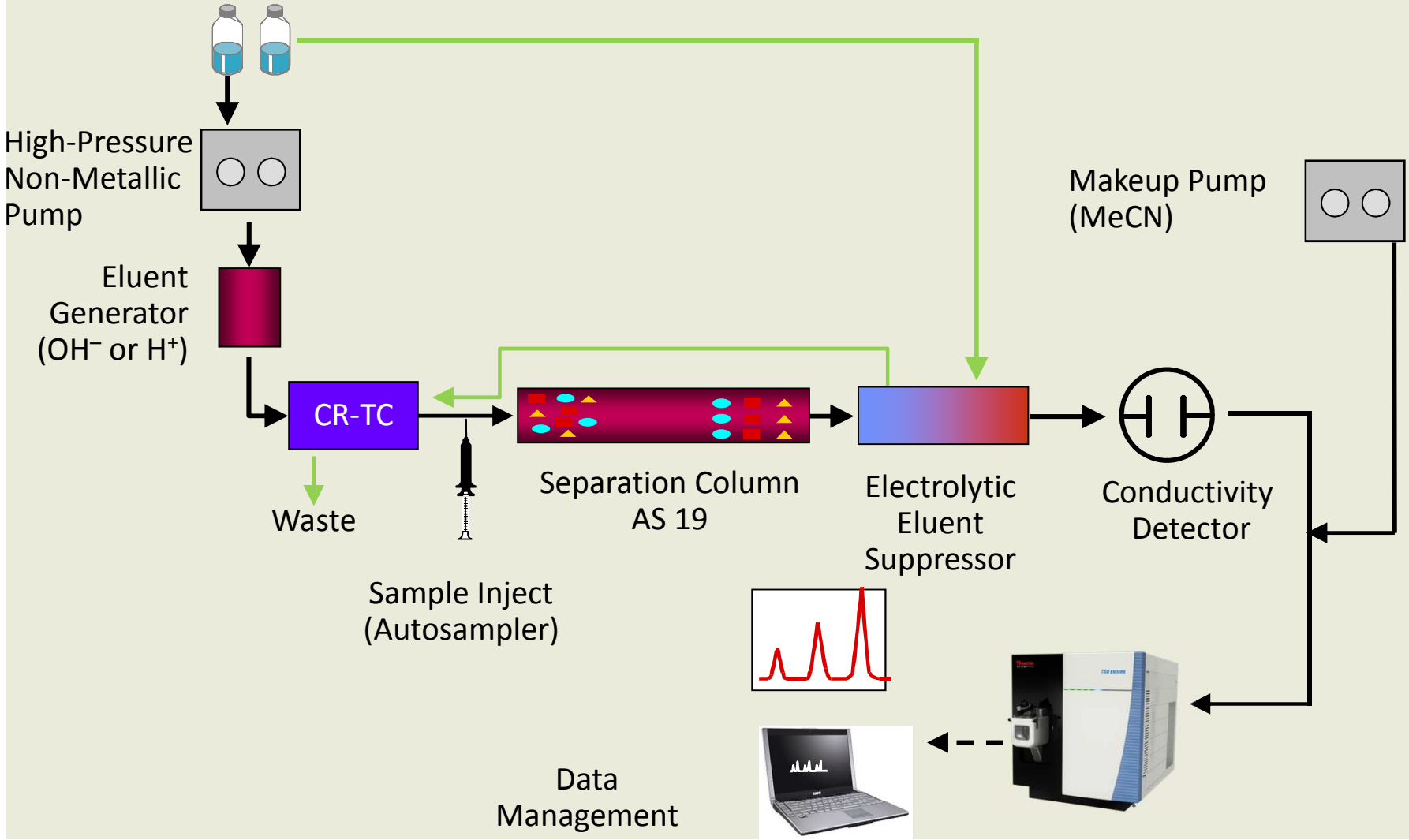


IC-MS/MS at Fera – today

- Currently evaluating a Thermo Scientific™ Dionex™ ICS 5000 system
- Fully integrated with Thermo Scientific™ TSQ Quantiva™ Triple Quadrupole MS
- Controlled through one software package – Thermo Scientific™ TraceFinder™ 3.2
- Multi-residue analysis of polar ionic pesticides in ‘QuPPe’ extracts

- Using new generation IC columns with 4 µm particle size
- MS tune optimised for low mass
- High sensitivity allowing lower volume injections
- Can change ion transfer tube whilst system under vacuum

IC-MS/MS Configuration



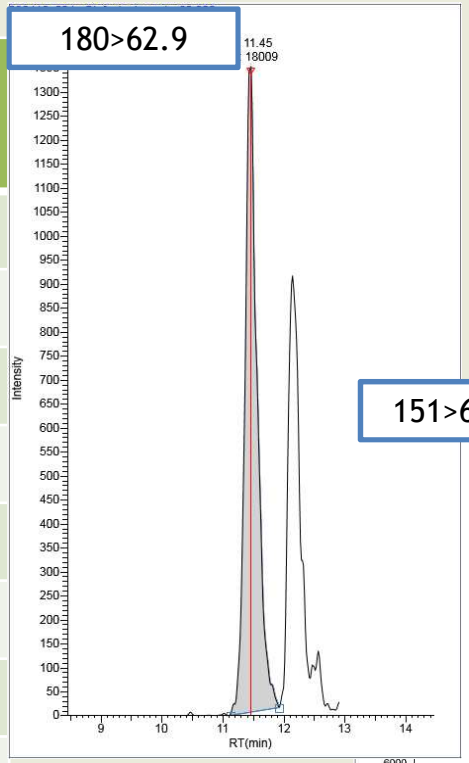
Validation – Cereals (Flour)

Compound	Concn (µg/kg)	Mean Recovery (n=5)	Mean % RSD
Perchlorate (IS)	10	95	6
	50	90	7
	100	92	9
Chlorate (IS)	10	93	5
	50	88	2
	100	87	4
Ethephon (IS)	10	95	11
	50	86	4
	100	85	4
Clopyralid	50	70	5
	100	89	6

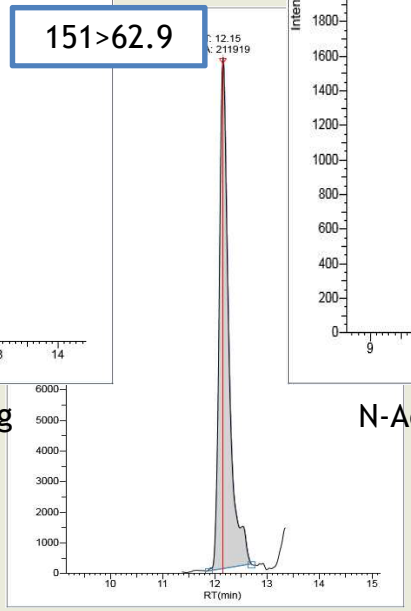
Compound	Concn (µg/kg)	Mean Recovery (n=5)	Mean % RSD
Fosetyl Al	200	60	4
	1,000	71	4
	2,000	72	2
Phosphonic acid	200	106	5
	1,000	94	4
	2,000	97	2
Cyanuric acid (IS)	50	75	31
	100	88	13

Glufosinate - Cereals (Flour)

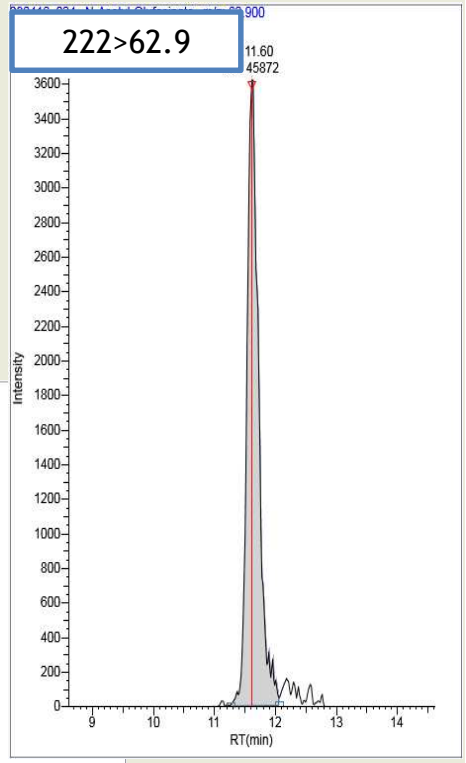
Compound	Concn (µg/kg)	Mean Rec (n=5)	Mean % RSD
Glufosinate (IS)	10	100	16
	50	109	11
	100	109	8
3-MPPA (IS)	10	106	17
	50	108	13
	100	111	7
N-Acetyl-Glufosinate (IS)	10	88	6
	50	88	9
	100	91	3



Glufosinate @ 50 µg/kg



3-MPPA @ 50 µg/kg

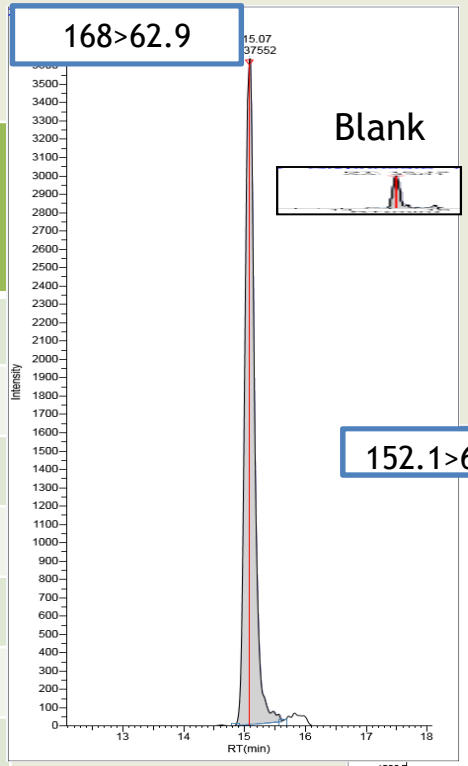


N-Acetyl-Glufosinate @ 50 µg/kg

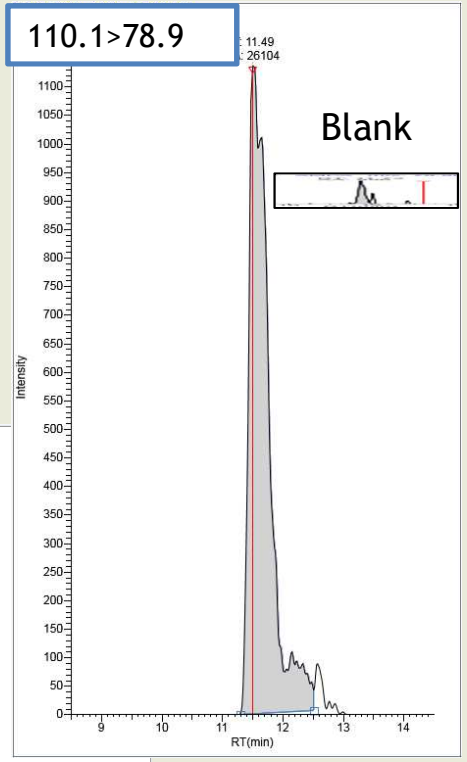
Glyphosate – Cereals (Flour)

Summary of validation results

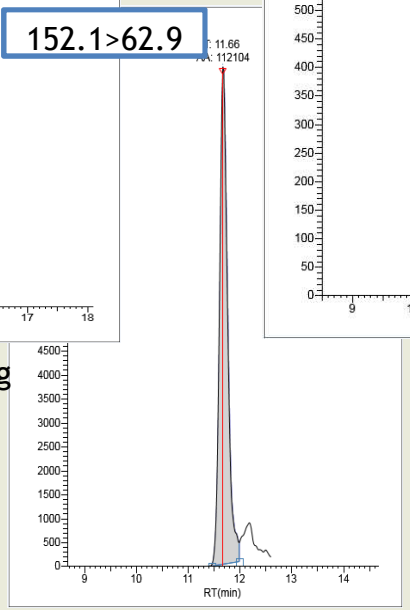
Compound	Concn (µg/kg)	Mean % Rec (n=5)	Mean % RSD
Glyphosate (IS)	10	112	15
	50	108	12
	100	111	7
AMPA (no IS)	10	92	22
	50	98	13
	100	97	3
N-Aectyl-AMPA (IS)	10	85	7
	50	82	10
	100	86	2



Glyphosate @ 50 µg/kg



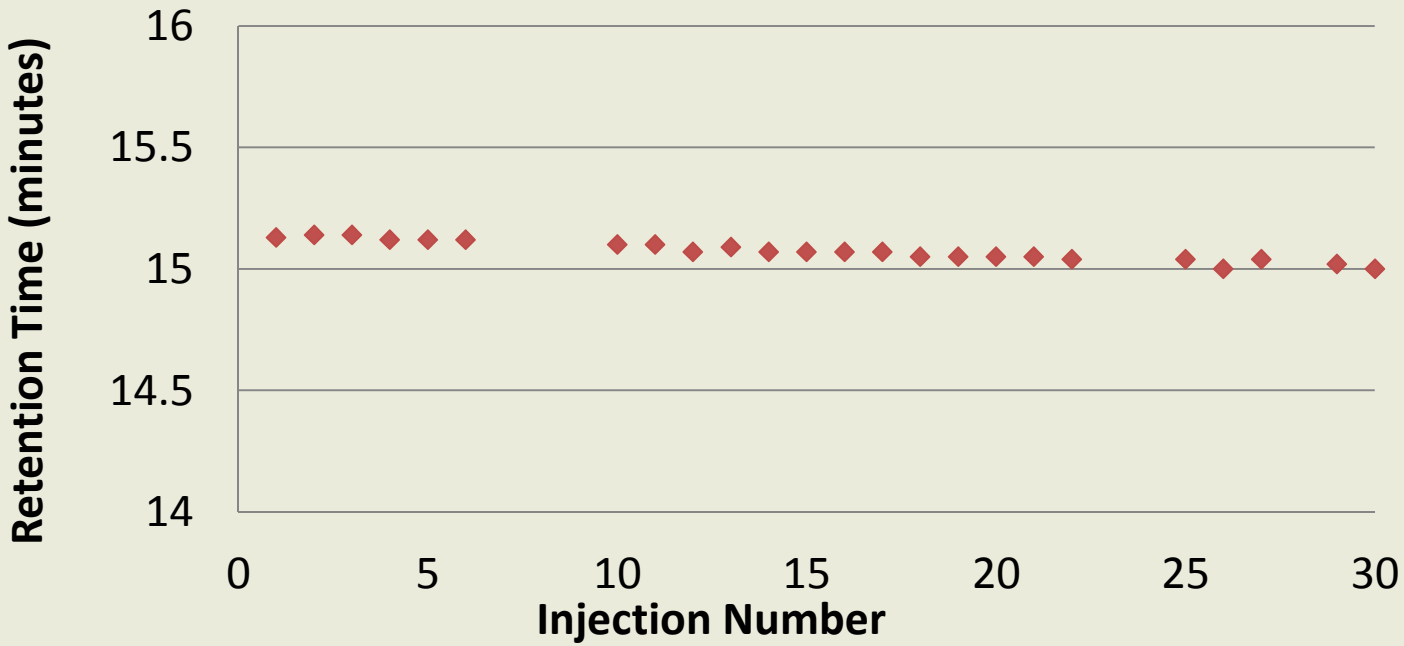
AMPA @ 50 µg/kg



N-Acetyl-AMPA @ 50 µg/kg

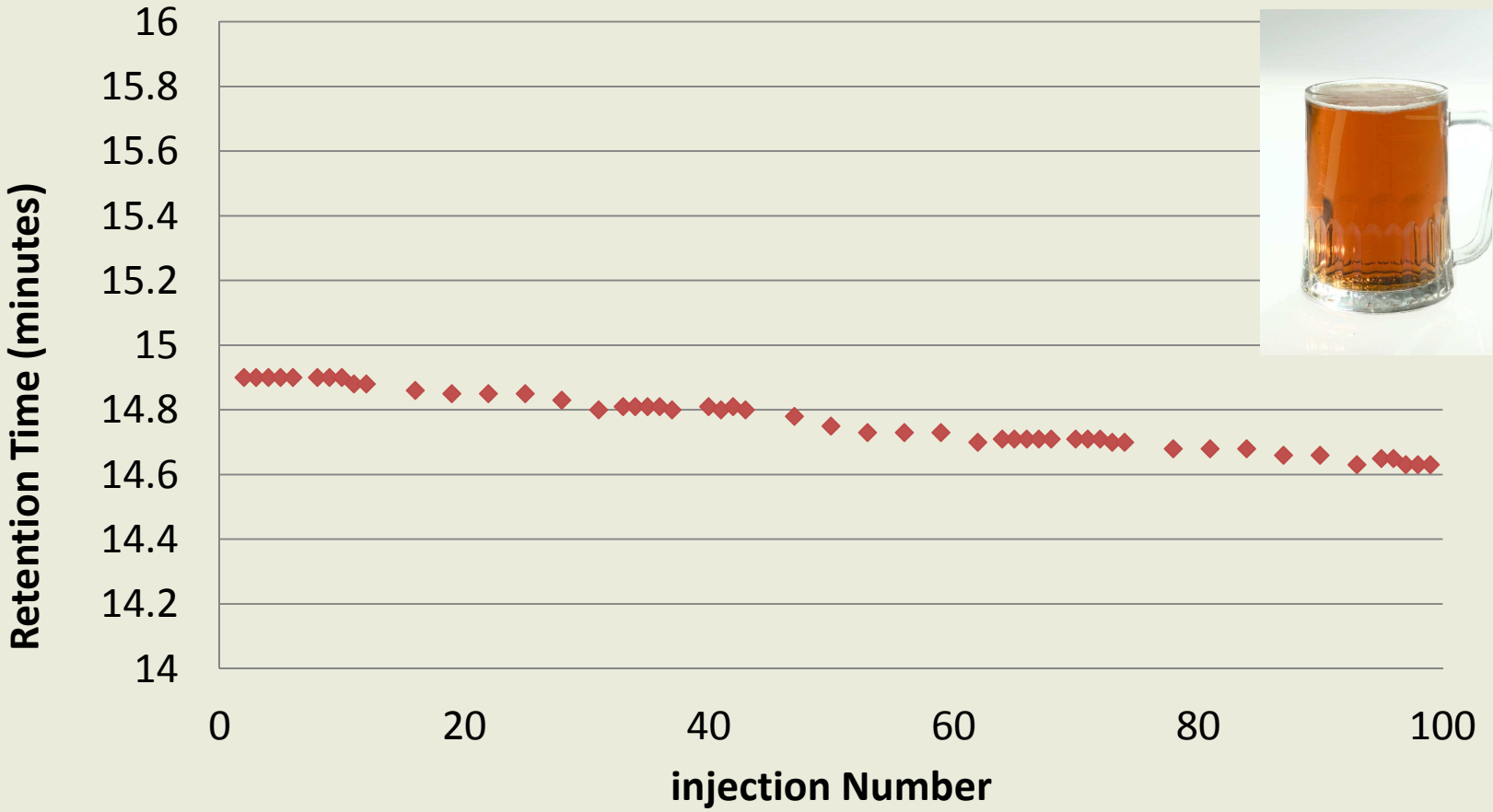
Glyphosate in cereals – retention time stability

- Over 30 injections in a cereals batch, retention time of glyphosate starts at 15.13 minutes and moves to 15.00 minutes



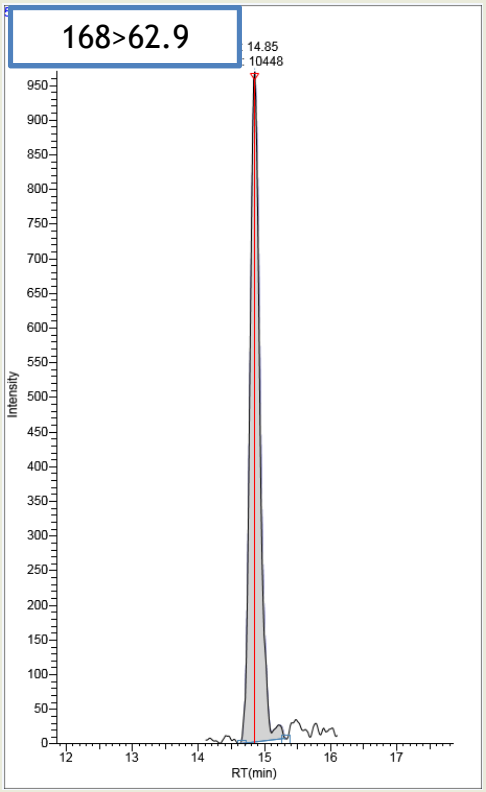
Glyphosate in beer – retention time stability

- Over 100 injections over 2.5 days, retention time of glyphosate starts at 14.90 minutes and moves to 14.63 minutes

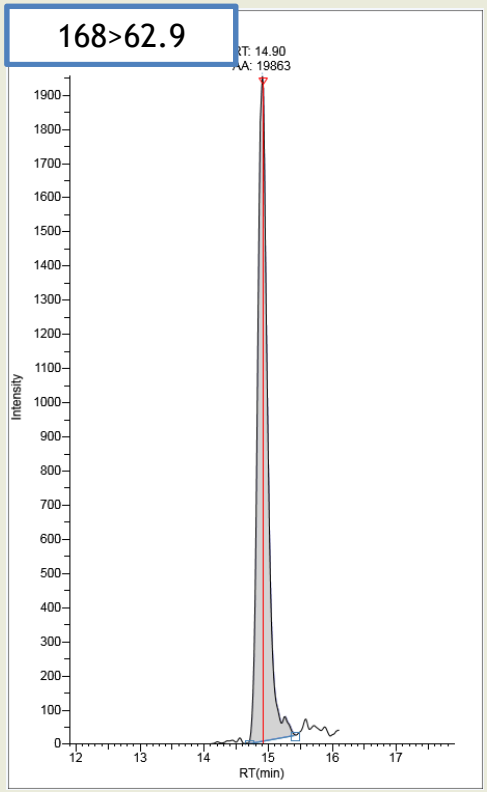


Glyphosate in beer – no extraction required!

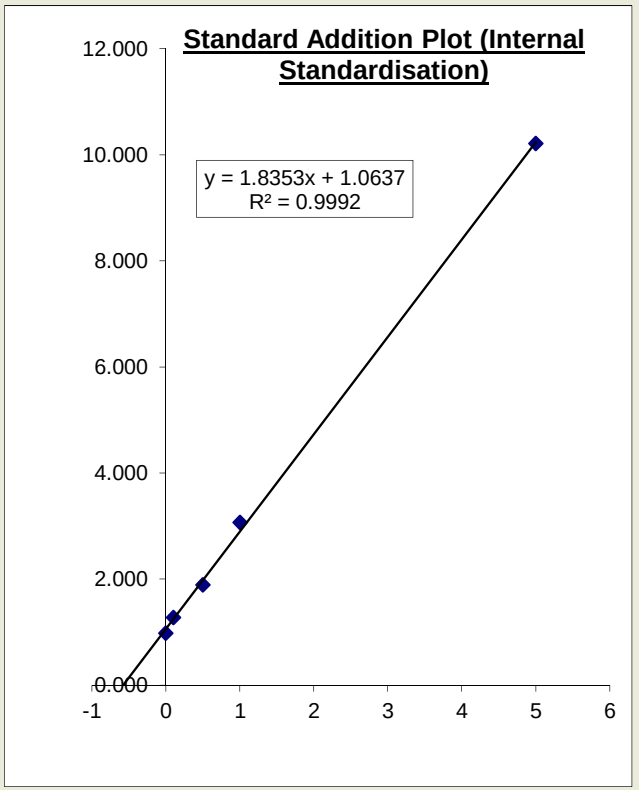
- Glyphosate incurred residue @ 0.58 µg/L



Glyphosate spike @ 0.5 µg/L



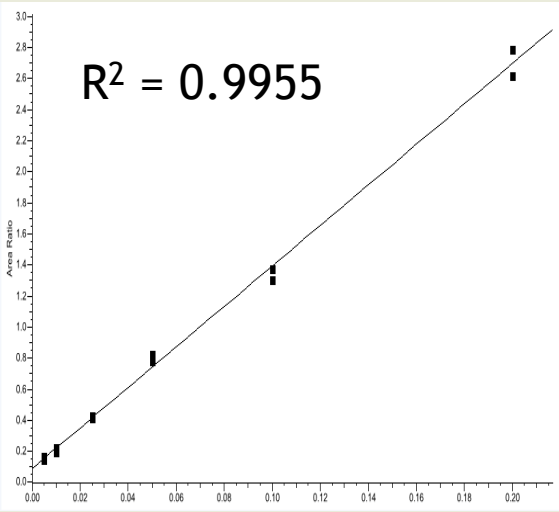
Calibration plot 0.1 – 5 µg/L spikes



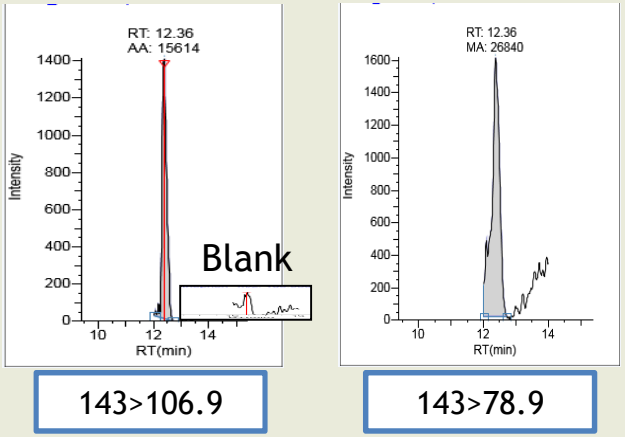
- 1/10 dilution with water and internal standard added

Ethephon –in grapes

Compound	Concn (µg/kg)	Mean Recovery (n=5)	Mean % RSD
Ethephon (IS)	10	114	17
	50	95	14
	100	102	10



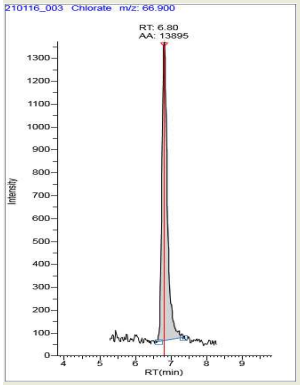
Ethephon @ 50 µg/kg in grape



143>106.9

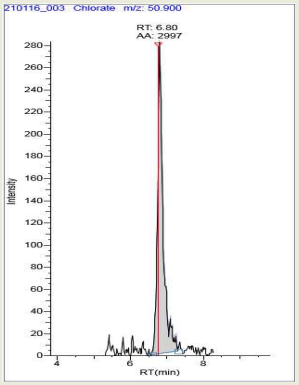
143>78.9

Chlorate in dairy products

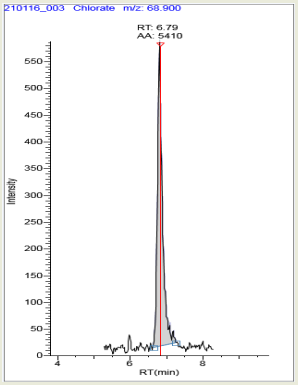


83.1>66.9

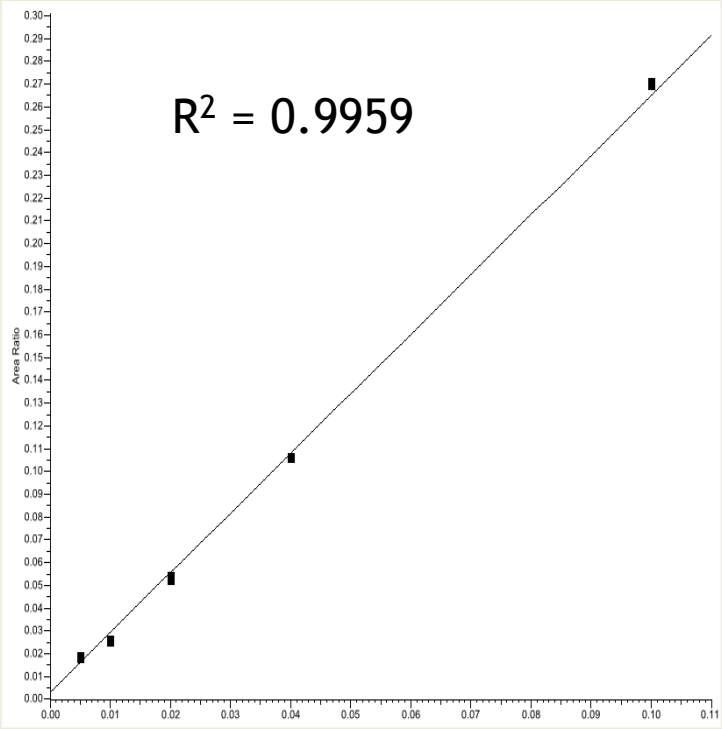
- Chlorate in dairy products @ 5 µg/kg in dairy products



83.1>50.9

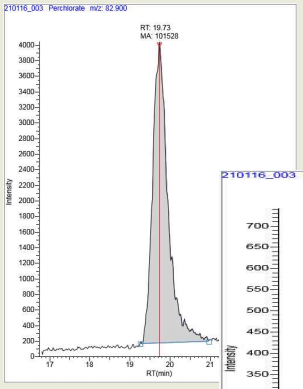


85.1>68.9

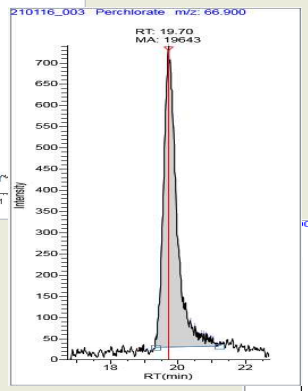


- Chlorate in dairy products calibration 5 - 100 µg/kg

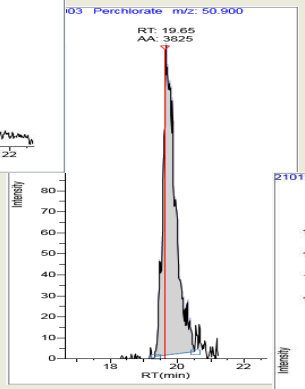
Perchlorate in dairy products



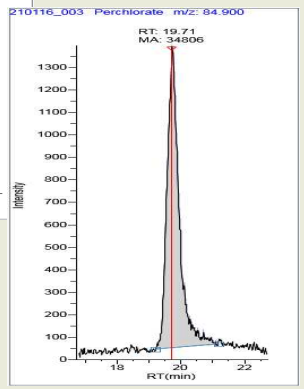
99>82.9



99>66.9

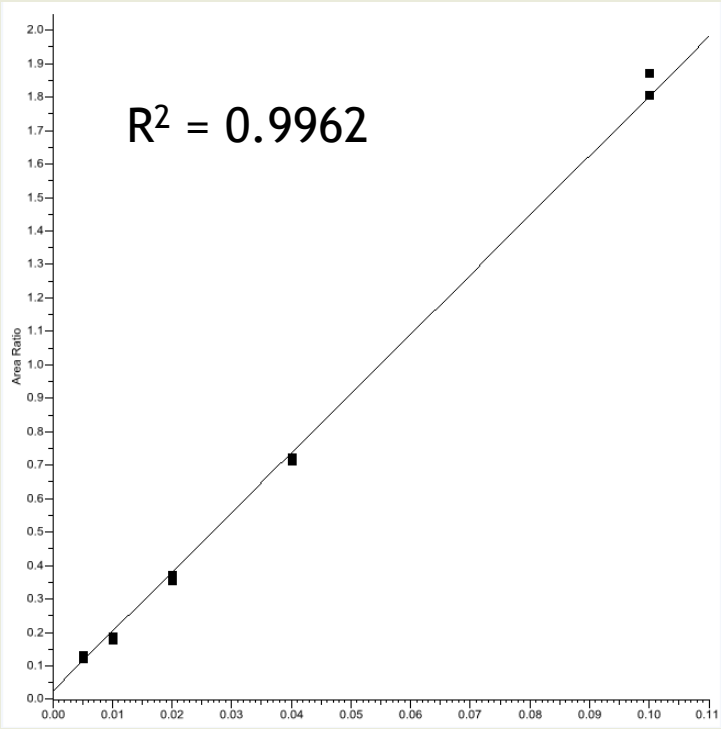


99>50.9



101>84.9

- Perchlorate in dairy products @ 5 µg/kg in dairy products

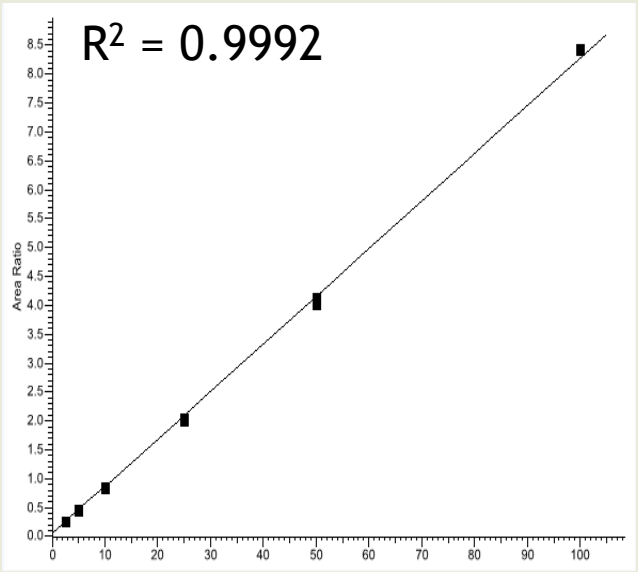


- Perchlorate in dairy products calibration 5 – 100 µg/kg

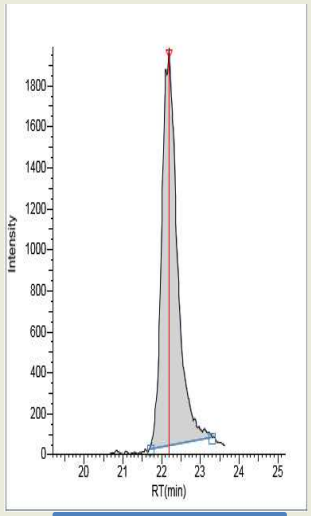
Validation – Perchlorate in Infant Food

- Perhlorate in organic infant food calculated at 2 µg/kg by standard addition (internally standardised)

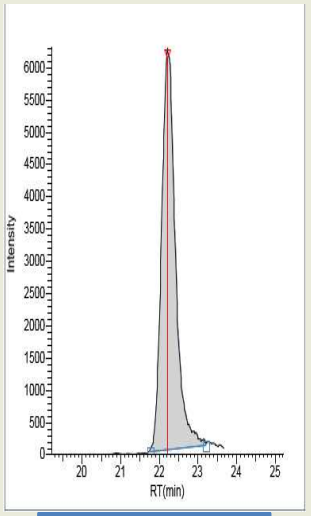
Compound	Concn (µg/kg)	Mean Recovery (n=5)	Mean % RSD
Perchlorate (IS)	7	116	2
	12	115	4
	52	104	1



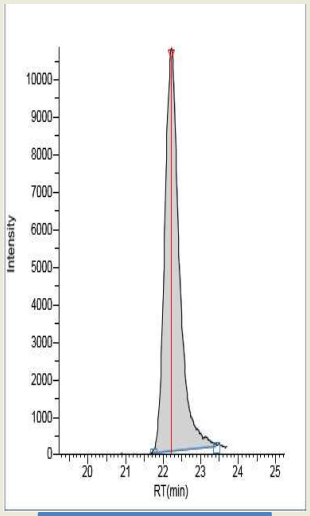
Blank (peak area 53,477)



Spike @ 5 µg/kg (peak area 154,418)

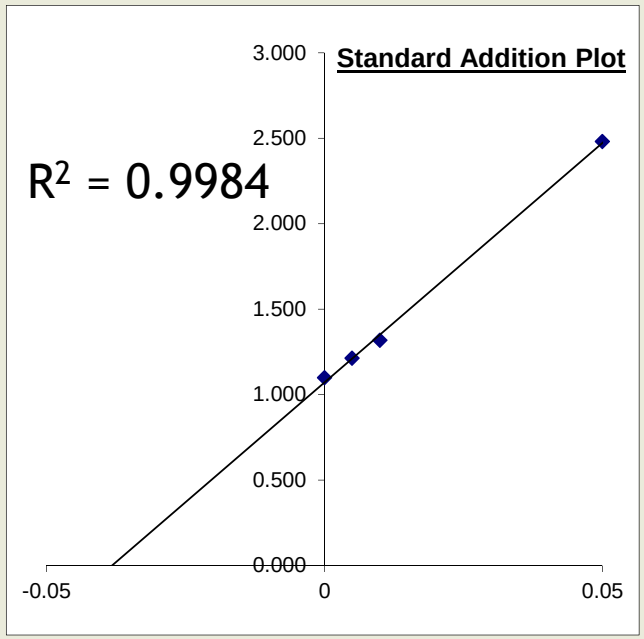


Spike @ 10 µg/kg (peak area 286,785)

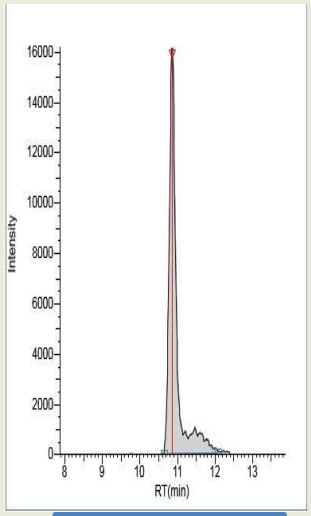


Danger! Chlorate in Infant Food

- Chlorate in organic infant food calculated at 38 µg/kg by standard addition (internally standardised)

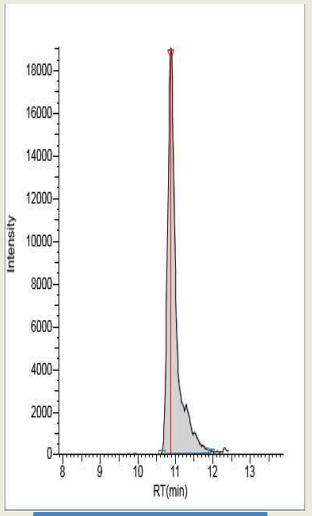


Blank
(peak area 215,734)



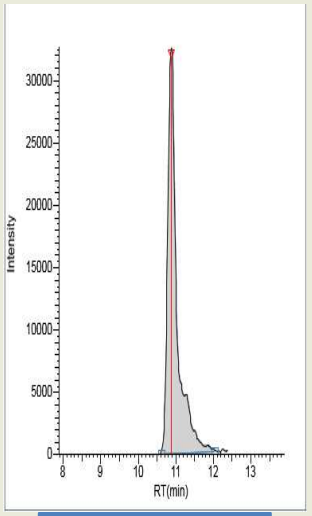
85.1>66.9

Spike @ 10 µg/kg
(peak area 301,9685)



85.1>66.9

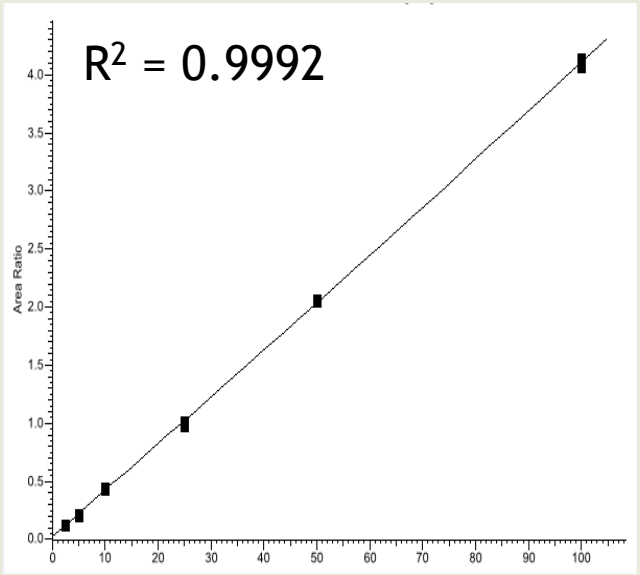
Spike @ 50 µg/kg
(peak area 555,594)



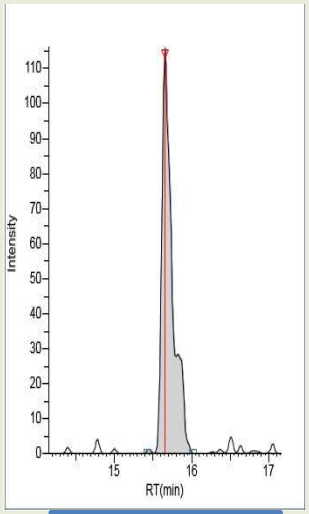
85.1>66.9

Validation – Glyphosate in Infant Food

Compound	Concn (µg/kg)	Mean Recovery (n=5)	Mean % RSD
Glyphosate (IS)	5	110	5
	10	120	12
	50	102	4

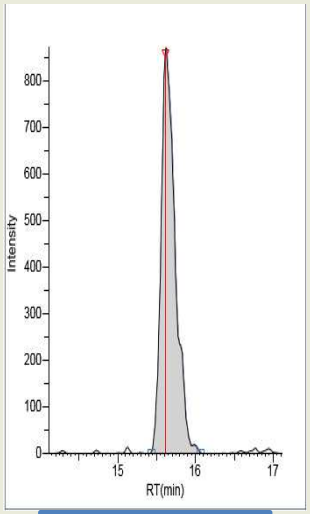


Blank (peak area 1,100)



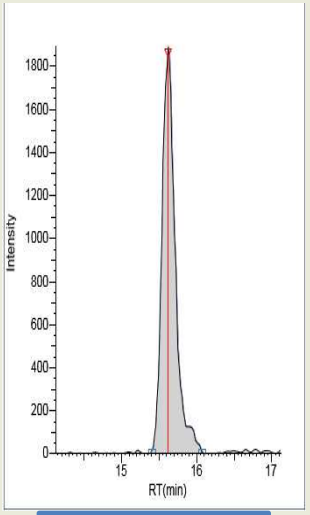
168>62.9

Spike @ 5 µg/kg (peak area 11,100)



168>62.9

Spike @ 10 µg/kg (peak area 23,000)



168>62.9

Summary

- Currently have an in-house validated method for 13 polar pesticides in two commodity groups
- Chlorate and perchlorate QuPPe method in routine use
- Infant food more challenging but still a work in progress with validation data for both glyphosate and perchlorate
- Good sensitivity and selectivity for validated pesticide/commodity combinations

Acknowledgements

- To Jonathan Guest and Mike Dickinson at Fera for their help during this work and the numerous others that have had brief interludes with IC-MS at Fera over the years

- To Jonathan Beck, Frans Schoutsen and Richard Fussell at Thermo Fisher Scientific for support in developing applications in this area

Any questions?